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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, 15/63, 15/70, 15/79, C07K 14/705	A1	(11) International Publication Number: WO 97/08316 (43) International Publication Date: 6 March 1997 (06.03.97)
(21) International Application Number: PCT/US96/13805 (22) International Filing Date: 27 August 1996 (27.08.96) (30) Priority Data: 60/003,020 31 August 1995 (31.08.95) US (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): DUONG, Le, T. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). NUTT, Elka, M. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). RODAN, Gideon, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: MOUSE INTEGRIN SUBUNITS (57) Abstract The full-length mouse $\beta 3$ integrin has been cloned and sequenced. A new form of $\beta 3$ integrin ($\beta 3$ -trunc) also has been cloned and sequenced.		

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TITLE OF THE INVENTION
MOUSE INTEGRIN SUBUNITS

DESCRIPTION OF THE INVENTION:

5 This invention relates to a new mouse vitronectin receptor subunit $\beta 3$ ($\beta 3$ -trunc), the full length mouse vitronectin receptor, their nucleic acids, and to assays using these receptors. Additionally this invention includes soluble integrins which lack transmembrane and cytoplasmic domains.

10

BACKGROUND OF THE INVENTION

 Integrins are transmembrane glycoproteins that mediate cell-cell and cell-matrix interactions. They contain two subunits, α and β , which are joined in a non-covalent complex. There are numerous α and β subunits known. Alpha subunits show some homology with other alpha subunits and beta subunits tend to show homology with other beta subunits, however, the alpha subunits tend to be quite distinct from beta subunits.

15 Osteoclasts are the primary cells responsible for bone resorption. Osteoclasts migrate to the area of the bone to be absorbed, and then attach to the bone. Adhesion molecules, including integrins, are believed to be involved in the processes of migration and attachment.

20 Recent studies have shown that both mature osteoclasts and tissue culture generated osteoclast-like cells highly express the vitronectin integrin receptor $\alpha_v\beta 3$. The $\alpha_v\beta 3$ integrin receptor recognizes the tripeptide Arg-Gly-Asp (RGD), found in many bone matrix proteins, and thus is thought to be involved in the attachment processes. However, there is no direct evidence that $\alpha_v\beta 3$ mediates
25 osteoclast attachment to bone *in vivo*.

30

 Partial sequence of the mouse $\beta 3$ cDNA was previously reported by Cieutat, *et al.*, 1993 *Biochem. Biophys. Res. Comm.* 193:771-778. Cieutat *et al.*, cloned $\beta 3$ from mouse kidney RNA using

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RT/PCR and human primers. This published sequence did not have the N-terminus and the last 4 amino acids at the C-terminus.

There are presently two types of screens for the $\alpha_v\beta_3$ ligands as an inhibitor for bone resorption: a binding assay based on human recombinant $\alpha_v\beta_3$ integrin and a functional assay based on rodent osteoclasts. To exclude the possibility of species-based potency differences in ligand interaction with the $\alpha_v\beta_3$ integrin, it would be desirable to develop an assay which uses the β_3 integrin subunit from a mouse osteoclast.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to the full length mouse β_3 integrin subunit (β_3), nucleic acids encoding it, and to processes for cloning it. Another aspect of this invention is a novel form of the β_3 integrin subunit, referred to as β_3 -trunc, which lacks the transmembrane and cytoplasmic domains, to nucleic acids encoding it, and to processes for producing it. Another aspect of this invention is the use of these integrins in assays to identify novel compounds which inhibit the bone absorption process.

Yet another aspect of this invention is a soluble ligand-binding integrin which, like other soluble receptors, suppresses the interaction of the full length integrins with their ligands. The main signal transduction pathway mediated by the a membrane bound integrin is transduced through the cytoplasmic domain of the β subunit. A soluble receptor, which has an intact binding domain but lacks the cytoplasmic domain, will suppress or compete with the normal signals mediated by the wild type receptor.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1. is the complete sequence of the mouse β_3 integrin (2.3 kb) cloned from a osteoclast cDNA library. The "ATG" initiation codon begins at position 164 and both a "TAA" and a "TGA" stop codons are seen starting at position 2525.

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Fig. 2 is the cDNA of the mouse $\beta 3$ -trunc. The "ATG" initiation codon begins at position 164.

Fig. 3 is the amino acid sequence of mouse $\beta 3$ -trunc. This sequence shows the corresponding amino acids, including untranslated regions. Asterisks denote stop codons. As shown in Figure 5, the open reading frame begins with the "Met" at position 55, and ends with the "Ala" at position 782.

Fig. 4 is the amino acid sequence of the full-length mouse $\beta 3$. This sequence shows corresponding amino acids, including untranslated regions. Asterisks denote stop codons. As shown in Figure 5, the open reading frame begins with the "Met" at position 55, and ends with the "Thr" at position 841.

Fig. 5 is an amino acid sequence comparison between the mouse full-length $\beta 3$ (top line) and the mouse $\beta 3$ -trunc (lower line).

Fig. 6 are gels showing the expression of mouse full-length $\beta 3$ and $\beta 3$ -trunc in osteoclast-like cells in the mouse co-culture system.

Fig. 7 are gels demonstrating the regulation of both $\beta 3$ and $\beta 3$ -trunc by 1,25-dihydroxy Vitamin D₃.

Fig. 8 are gels showing the expression of $\beta 3$ and $\beta 3$ -trunc in various tissues.

Fig. 9 are diagrams of the mouse $\beta 3$ and $\beta 3$ -trunc genes and the proteins encoded.

As used in the specification and claims, the following definitions shall apply:

"Free from associated mouse nucleic acid" - physically separated from mouse nucleic acid (DNA or RNA) which either (i) mouse $\beta 3$ nucleic acid or (ii) mouse $\beta 3$ -trunc nucleic acid.

"Free from associated mouse DNA"-- physically separated from mouse DNA which is not either (i) mouse DNA encoding $\beta 3$ integrin or (ii) DNA encoding truncated $\beta 3$ integrin.

"Substantially pure"-- a protein or nucleic acid is "substantially pure" when the amount of other protein or nucleic acid present in a sample is less than about 5% of the sample by weight.

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Thus one aspect of this invention is nucleic acids which encode the full length mouse $\beta 3$ integrin, said nucleic acid being free from associated mouse nucleic acid. Preferably the nucleic acid is a DNA. A preferred type of DNA is cDNA, and a particularly preferred cDNA is that shown in Figure 1.

Partial sequence of the mouse $\beta 3$ cDNA was previously reported by Cieutat, *et al.*, 1993 *Biochem. Biophys. Res. Comm.* 193:771-778, which is hereby incorporated by reference. Cieutat *et al* cloned $\beta 3$ from mouse kidney RNA using RT/PCR and human primers. This published sequence did not have the N-terminus and the last 4 amino acids at the C-terminus. One aspect of this invention comprises a complete sequence of the mouse $\beta 3$ integrin (2.3 kb) cloned from a osteoclast cDNA library, free from associated mouse cDNA, or which is substantially pure. This is presented in Figure 1. The sequence of $\beta 3$ was derived from the cDNA sequence of clone 9A (from 5'-end to base 2028) and the PCR sequence of a fragment encoding the last 363 bases at the 3'-end.

Another aspect of this invention is the complete, full-length $\beta 3$ peptide, free from associated mouse peptides, or substantially pure which is shown in Figure 4. Substantially pure mouse full-length $\beta 3$ is another aspect of this invention.

Mouse $\beta 3$ shows 86% homology with the human $\beta 3$ at the DNA level, 90% overall homology in the amino acid sequence, 90% and 100% homology in the ligand binding domains (residues 109 - 171 and residues 204 - 229, respectively), 97% homology in the transmembrane domain and 100% identity in the cytoplasmic tail. This high homology is consistent with the quantitative similarity in the binding of ligands to human and mouse $\alpha v \beta 3$.

Another aspect of this invention are vectors which comprise the full length mouse $\beta 3$ nucleic acids, preferably cDNA and to host cells transformed with these vectors. Preferred host cells are embryonic kidney cells. This invention also includes the method of making full length $\beta 3$ by transforming a host cell with a vector

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comprising full length mouse $\beta 3$ DNA and harvesting the $\beta 3$ so produced.

Characterization of the truncated mouse $\beta 3$ cDNA ($\beta 3$ -trunc):

5 Another aspect of this invention is nucleic acids which encode a truncated mouse $\beta 3$ ($\beta 3$ -trunc) peptide, free from associated mouse nucleic acids, or which are substantially pure. A preferred form of $\beta 3$ -trunc DNA is cDNA; a particularly preferred cDNA is that shown in Figure 2.

10 Another aspect of this invention is the $\beta 3$ -trunc peptide, free from associated mouse peptides, or substantially pure. This is shown in Figure 3 and Figure 9. Mouse $\beta 3$ -trunc, which includes 5'-untranslated region (163 bp), 5'-coding region of the extracellular domain of $\beta 3$ (up to base 2028 or residue 676) and a diversified 3'-coding region. Interestingly, the diversified 3'-coding region includes an in-frame addition of 43 amino acids, followed by a long 3'-untranslated sequence (1.2 kb). From homology analysis, this diversified 3'-sequence shows no significant homology with any known gene. The protein encoded by the $\beta 3$ -trunc gene contains the entire ligand binding and cysteine-rich domains, but lacks the transmembrane and cytoplasmic domains.

20 The expression of $\beta 3$ -trunc and its regulation in the co-culture-derived osteoclasts was investigated. Northern analysis of the co-culture, with either a 5'-probe or a 3'-specific $\beta 3$ -trunc probe, reveals that the osteoblastic MB 1.8 cells do not express $\beta 3$ or $\beta 3$ -trunc (see Figure 6). However, the expression of both forms is highly enriched in the partially purified preparation of osteoclasts from the co-culture. The 5'-probe hybridizes to a major mRNA product at 6.5 kb and several minor forms of 2-4 kb. The $\beta 3$ -trunc specific probe detects 25 a major mRNA product at 3 kb and two minor mRNA products at 2 and 30 4 kb. Generation of osteoclasts in the co-culture system depends on the presence of 1,25-dihydroxy Vitamin D₃ (1,25(OH)₂D₃). Both forms of $\beta 3$ integrin were up-regulated by 1,25(OH)₂D₃ treatment of the co-culture system as shown in Figure 7.

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Murine tissue distribution reveals different patterns of expression for $\beta 3$ and $\beta 3$ -trunc. This is demonstrated in Figure 8. Full length $\beta 3$ is expressed in spleen>lung>liver, with a very minor amount of $\beta 3$ messages (6.5kb) detected in other tissues. In contrast, $\beta 3$ -trunc (2-4 kb) messages are expressed in heart>skeletal muscle>brain>lung.

Since $\beta 3$ -trunc lacks the transmembrane and cytoplasmic domains, it can be considered a soluble ligand binding integrin. This represents the first such soluble integrin. Thus another aspect of this invention is an integrin which lacks the transmembrane and cytoplasmic domains. Such an integrin is able to circulate throughout the organism. Its physiological role appears to be suppression of the signaling pathway mediated by the full length $\beta 3$ integrins interaction with their ligands. Integrin-ligand signals are generally transmitted to the cytoplasm by a mechanism involving the cytoplasmic domain. However, when a ligand binds to $\beta 3$ -trunc, which lacks such a domain, the signal would not reach the cytoplasm. Therefore, the soluble ligands can act as negative regulators, tying up ligand without signaling the cell.

Assays

Another aspect of this invention are novel assays. The novel assays of this invention are to identify inhibitors of human $\alpha_v\beta 3$ receptors. Such inhibitors would be useful in a variety of disease conditions including diseases associated with bone resorption such as osteoporosis. Generally, potential inhibitors are first screened for their ability to bind to recombinant human $\alpha_v\beta 3$ receptors using an assay such as the one set forth in Example 2. Further *in vitro* testing of the potential inhibitor, however, generally occurs using mouse or other rodent cell systems. It is not uncommon for the same potential inhibitor to display different responses in the two systems, and until now the investigator would not be able to determine if the differences were due to the effect of the different species' receptors or to actual *in vitro* activity.

Thus, in one aspect of this invention, a potential inhibitor to osteoclast formation is placed into contact with either mouse full length

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5 $\beta 3$ or mouse $\beta 3$ -trunc, and its ability to bind is measured. The binding may be measured by any known means, such as by measuring the displacement of a compound known to bind to $\beta 3$, such as echistatin. This information can be used to better assess the activity of the potential inhibitor in an *in vitro* assay.

10 By means of example only, if a potential inhibitory compound were found to bind well to human $\alpha_v\beta 3$ in the recombinant $\alpha_v\beta 3$ assay, but exhibited less inhibitory activity than expected in the mouse *in vitro* assay, one could determine whether the decrease in expected activity was due to the compound's inability to bind efficiently to the mouse integrin or whether the decreased activity was a true reflection of the compound's *in vitro* activity, by performing a mouse $\beta 3$ or $\beta 3$ -trunc assay.

15 The following non-limiting Examples are presented to further illustrate the invention.

EXAMPLES

General techniques

20 First-Strand cDNA synthesis kit and QuickPrep mRNA Purification Kit were from Pharmacia. Lamda ZAP II cloning kits were from Stratagene. Mouse tissue mRNA blots were purchased from Clontech. Hybond-N filters were from Amersham. Restriction enzymes were from various sources: BioLabs, Promega and Stratagene.
25 Tissue culture media were from Gibco. Fetal bovine serum was obtained from JRH Bioscience.

EXAMPLE 1

30 Strategy for isolating cDNA clones for the mouse $\beta 3$ subunit

Generation of a mouse $\beta 3$ cDNA probe (m $\beta 3$ probe): This probe was generated using the following degenerate oligonucleotide primers:

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5'-primer:

CCA AGC TTG AC(A/C) T(G/C)T ACT A(C/T)C T(G/T)A TGG A

3'-primer:

5 CCC TCG AGA A(A/G)T (C/T)GT CGC A(C/T)T CGC A(A/G)T A

The primers were designed based on a sequence which is highly conserved among all integrin β subunits (Ramaswamy & Hemler, 1990, *EMBO J.* 9: 1561-1568, which is incorporated by reference).

10 Using polymerase chain reaction, a cDNA fragment of the $\beta 3$ subunit was cloned from a cDNA library prepared from mouse osteoclasts. The identity of this m $\beta 3$ probe was confirmed by sequence analysis to be homologous to the published human $\beta 3$ sequence (Frachet *et al.*, 1990 *Mol. Biol. Rep.* 14:27-33, which is hereby incorporated by reference.).

15

Construction of a λ ZAP mouse osteoclast cDNA library (λ ZAP-OC):

The cDNA library was constructed from 5 μ g polyA(+) RNA prepared from osteoclasts, which were generated from a co-culture of osteoblastic MB 1.8 cells and mouse bone marrow cells in the presence of 1,25-dihydroxy Vitamin D₃ (1,25(OH)₂D₃). Methods for generation and isolation of mouse osteoclasts from culture were performed as described by Tanaka, *et al.*, 1991 *J. Bone Min. Res.* 6: S148, which is hereby incorporated by reference. The construction of this library was carried out according the instructions provided by the manufacturer, Stratagene (Lambda ZAP II Cloning Kits - 236611). Random pd(N)₆ primers were used for the first strand cDNA synthesis.

30 Screening for mouse $\beta 3$ clones: Mouse $\beta 3$ cDNA clones were isolated by screening the primary λ ZAP-OC library (0.5×10^6 pfu), using the m $\beta 3$ probe. Sixteen positive clones were isolated and rescued into pBluescript phagemid according to the manufacturer's protocol (Stratagene). These clones were initially characterized by restriction digestion with EcoRI to estimate the size of cDNA inserts. Clone 9A

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was found to be the largest (3.5 Kb) and was subsequently characterized by sequence analysis.

5 Cloning of 3'-cDNA fragment of mouse β 3 by PCR: Clone 9A encodes for the entire sequence of mouse β 3-trunc, which lacks only 121 amino acids (363 bp) from the expected C-terminus of β 3-full, based on the published human β 3 sequence. Therefore, the rest of the 3'-cDNA fragment was cloned by PCR. The following primers were used:

10 5'-primer (from BstEII site of clone 9A):
TAA GGA CAG CCT CAC CGT CCA GGT

15 3'-primer (based on the human sequence):
TCA TTA AGT CCT CGG TAC GTG ATA TTG GTG

Full length mouse β 3 cDNA was then constructed by ligating at the BstEII site between the clone 9A-derived 5'-fragment and the PCR clone-derived 3'-fragment.

20 RNA isolation and Northern blot analysis: Total cellular RNA was isolated by guanidine isothiocyanate and phenol extraction (Chomczynski & Sacchi, 1987, *Anal. Biochem.* 162:156-159.). Ten μ g of total RNA was separated using formaldehyde-agarose gel electrophoresis, followed by transfer onto nylon filters (Hybond-N; Amersham). Poly A(+) RNA was prepared using QuickPrep mRNA Purification Kit (Pharmacia). Mouse tissue blots were purchased from Clontech. Mouse β 3 specific probe was generated from the 5'-fragment of clone 9A using the EcoRI and BstEII sites. This probe can recognize both β 3 full length and β 3-trunc. Mouse β 3-trunc specific probe was
25 generated from the 3'-fragment of clone 9A using the Not I and EcoRI
30 sites. Hybridizations were performed in 40% formamide, 5x SSC, 0.1% SDS, 0.1% ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA and 200 mg/ml sonicated salmon sperm DNA at 42°C, overnight, and washed two times

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(30 min) at 55°C in 0.1x SSC and 0.1% SDS. The filters were dried and exposed to XAR-2 films (Eastman Kodak, Rochester, NY).

EXAMPLE 2

5

Osteoclast Formation Assay:

Osteoclast formation was determined using the mouse bone marrow-derived osteoblast co-culture system, as described by Takahashi, *et al.*, 1988. In this assay, an osteoblastic cell line (MB1.8), established from neonatal mouse calvaria, were plated in 24-well culture dishes, at 10,000 cells per cm² in α -MEM containing 10% fetal bovine serum and 10 nM 1,25(OH)₂D₃. Balb/C male mice (six weeks old) were sacrificed under CO₂, and tibiae and femors were aseptically removed. The bone ends were cut off with scissors and the marrow cavity was flushed with 1 ml α -MEM by using a 27G needle. The bone marrow cells were then filtered through 70 μ m nylon mesh. Cells were centrifuged for 7 min. at 300xg and washed once with α -MEM and finally resuspended and aliquoted at 25,000 cells/cm² onto the MB1.8 cells in the 24-well culture dishes. Medium with 10 nM 1,25(OH)₂D₃ was replaced every two days. Potential inhibitors of osteoclast formation were added to the cultures at day 2 and at day 4. After 7 days, the cultures were fixed and stained for Tartrate-resistance acid phosphatase (Trap) activity, essentially as described in Takahashi, *et al.*, 1988. The formation of osteoclasts in this co-culture was quantitated as the number of multinucleated Trap(+) cells (with three or more nuclei) per well of a 24-well tissue culture plate.

Recombinant Expression of functional human integrin $\alpha_v\beta_3$:

cDNAs for human α_v and human β_3 were cloned into pR135 and pCDNAI-neo expression vectors, both of which use the CMV promoter but contain hygromycin or neomycin resistance markers, respectively. Using these selection markers, we established a stable human embryonic kidney 293 cell line that stably expresses high levels of recombinant human $\alpha_v\beta_3$ was established. Surface expression of the

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receptor in this 293($\alpha_v\beta_3$) cell line were characterized using northern analysis, surface radioiodination followed by immunoprecipitation. In addition, the number of $\alpha_v\beta_3$ integrin receptors on the cell surface was estimated to be 1×10^6 receptor per cell, based on specific binding of $\alpha_v\beta_3$ to radio-iodinated echistatin.

Using the 293($\alpha_v\beta_3$) cell line, two different assays were developed for screening inhibitors of the integrin $\alpha_v\beta_3$: echistatin binding assay (EIB) and vitronectin cell attachment assay (VNADIN), below.

Echistatin Binding assay (EIB):

The membrane fraction of 293($\alpha_v\beta_3$) was solubilized in 100 mM octyl glucoside and the membrane protein extract is used in radio-iodinated echistatin binding. Binding buffer is 1% bovine serum albumin, 50 mM Tris-HCl (pH 7.2), 150 mM NaCl, 1mM CaCl_2 and 1mM MgCl_2 . Membrane extract is incubated with radioiodinated echistatin (50,000 cpm), in the absence (total binding) or in the presence of unlabeled echistatin (specific binding) or in the presence of test compounds. Incubation period is 1 hour at room temperature. Specific echistatin bound proteins are filtered through a membrane using a Skatron Cell Harvester system.

Vitronectin Cell Attachment Assay (VNADIN):

96-well plates are coated with human vitronectin 293($\alpha_v\beta_3$) cells are lifted in trypsin/EDTA and washed in serum-free media. Cells are resuspended in attachment medium (Hank's balance salt containing BSA (1mg/ml) and CaCl_2 (2mM). Cells are then allowed to attach to vitronectin-coated wells for 1 hr at 37°C, in the absence (total attachment) or in the presence of tested compounds. Non-adhered cells are then removed by gently washing the wells with phosphate buffered saline.

The number of adhered cells can be quantitated by determining the relative levels of glucosaminidase activity overnight. The enzyme substrate solution is 3.75 mM p-nitrophenyl-N-acetyl- β -D-

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- glucosaminide in 0.1 M citrate buffer (pH 5.0) and 0.25% Triton X-100. The plates are incubated in the dark, room temperature, overnight. The color reaction is then developed by addition of 50 mM glycine, 5 mM EDTA at pH 10.5. Absorbance at O.D. 405 nm is
- 5 determined and the number of cells can be quantitated using a standard curve of cells.

Assays using mouse $\beta 3$:

- Essentially the same procedure is followed as described
- 10 above to create a human embryonic kidney 293 cell line expressing either full-length mouse $\beta 3$ or mouse $\beta 3$ trunc. The EIB and/or VNADIN assays are then performed substantially as described, substituting the mouse $\beta 3$ or mouse $\beta 3$ -trunc expressing cells.

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WHAT IS CLAIMED IS:

1. A nucleic acid encoding full-length mouse $\beta 3$ integrin subunit, said nucleic acid being free from associated mouse nucleic acid.
5
2. A nucleic acid according to Claim 1 which is DNA.
3. DNA according to Claim 2 which is substantially
10 pure.
4. DNA according to Claim 2 which is cDNA.
5. DNA according to Claim 4 which is shown in
15 Figure 1.
6. A vector comprising any of the nucleic acid of Claims 1-5.
7. A host cell comprising the vector of Claim 6.
20
8. A host cell according to Claim 7 which is embryonic kidney cells.
9. A method for making full length mouse $\beta 3$ subunit comprising transforming a host cell with a vector comprising mouse $\beta 3$ cDNA and harvesting the $\beta 3$ so produced.
25
10. Full-length mouse $\beta 3$ integrin, which is shown in
30 Figure 4, said mouse $\beta 3$ integrin being free from associated mouse proteins.
11. Substantially pure full length mouse $\beta 3$ integrin, which is shown in Figure 4.

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12. A nucleic acid encoding mouse integrin $\beta 3$ -trunc subunit, said nucleic acid being free from associated mouse nucleic acid.
13. A nucleic acid according to Claim 12 which is DNA.
14. DNA according to Claim 13 which is substantially pure.
15. DNA according to Claim 13 which is cDNA.
16. DNA according to Claim 15 which is shown in Figure 2.
17. A vector comprising any of the nucleic acids of Claims 12-16.
18. A host cell comprising the vector of Claim 17.
19. A method for making mouse $\beta 3$ -trunc comprising transforming a host cell with a vector comprising mouse $\beta 3$ -trunc cDNA and harvesting the $\beta 3$ -trunc so produced.
20. Mouse $\beta 3$ -trunc integrin, which is shown in Figure 3, said mouse $\beta 3$ -trunc integrin being free from associated mouse proteins.
21. A method to determine the ability a compound to bind to full-length $\beta 3$ or $\beta 3$ -trunc integrin comprising contacting the compound with either full length $\beta 3$ or $\beta 3$ -trunc and measuring the resultant binding.
22. A soluble integrin which comprises a binding domain, but which lacks a cytoplasmic domain and a transmembrane domain.

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23. A method for identifying a compound which binds to a soluble integrin which possesses a binding domain but which lacks a cytoplasmic domain and a transmembrane domain comprising contacting the compound with the soluble integrin and determining whether
- 5 binding occurs.

1/20

1 ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCTCG
51 AAATTAACCC TCACTAAAGG GAACAAAAGC TGGAGCTCCA CCGGTGGCGG
101 CCGCTCTAGA ACTAGTGGAT CCCCCGGCT GCAGGAATTC GCGCCGTCGA
151 CGCGGCGGAC AGGATGCCGAG CGCAGTGGCC GGGACAACTC TGGGCCGCTC
201 TGCTGGCGCT GGGGGCGCTG GCGGGCGTTG TTGTTGGAGA GTCCAACATC
251 TGTACCACAC GAGGCGTGAA CTCCTGCCAG CAGTGTCTGG CTGTGAGTCC
301 TGTGTGTGCC TGGTGCTCAG ATGAGACTTT GTCTCAGGGC TCACCCCGAT
351 GTAACCTGAA GGAGAACCTG CTGAAGGACA ATTGTGCTCC AGAGTCTATT
401 GAGTCCCCAG TCAGTGAGGC CCAGATCCTG GAGGCTAGGC CACTCAGCAG
451 CAAGGGCTCT GGAAGCAGCG CCCAGATCAC TCAAGTCAGC CCTCAGAGGA
501 TTGCCCTTCG ACTACGGCCA GATGATTCGA AGATCTTCTC ACTTCAAGTG
551 CGGCAGGTGG AGGATTACCC CGTGGACATC TACTACTTGA TGGACCTGTC
601 TTTCTCCATG AAGGATGATC TGTCCAGCAT CCAGACCCCTG GGTACCAAGT
651 TGGCCTCTCA GATGCGCAAG CTTACTAGCA ACCTTCGGAT TGGCTTTGGG

FIG. 1A

RECTIFIED SHEET (RULE 91)

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701 GCCTTCGTGG ACAAGCCTGT ATGCCCGTAC ATGTACATCT CCCCACCACA
751 GGCAATCAAA AACCCCTGTT ACAATATGAA GAATGCCTGC TTGCCCATGT
801 TTGGCTACAA ACACGTGCTG ACGCTAACCG ACCAGGTGTC CCGCTTCAAT
851 GAAGAAGTGA AGAAACAGAG CGTGTCCCGT AATCGAGATG CCCCAGAGGG
901 CGGCTTTGAC GCCATCATGC AGGCTACAGT ATGTGATGAA AAAATTGGCT
951 GGAGGAATGA CGCATCCCAT TTGCTAGTGT TTACCACGGA TGCCAAGACC
1001 CATATTGCCC TGGATGGAAG ACTGCGAGGC ATTGTCCCTGC CCAATGATGG
1051 GCACTGTCAC ATTGGCACCG ACAACCACTA CTCTGCCCTCC ACTACCATGG
1101 ACTACCCATC TCTGGGGCTG ATGACTGAGA AACTATCCCA GAAAACATT
1151 AACTTGATCT TTGCAGTGAC TGAAAATGTC GTCAGCCTTT ACCAGAAATTA
1201 TAGTGAGCTC ATTCCTGGGA CCACAGTGGG AGTCCCTGTCT GATGACTCAA
1251 GCAACGTCCT CCAGCTGATT GTTGATGCTT ACGGAAAAT CCGCTCTAAA
1301 GTGGAGCTGG AAGTACGTGA CCTGCCGGAA GAACTGTCAC TGTCCCTCAA
1351 TGCCACCTGC CTCAACAACG AGGTTATCCC GGGCCTCAAG TCTTGTGTGG

FIG. 1B

RECTIFIED SHEET (RULE 91)

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1401 GCCGCAAGAT TGGAGACACG GTGAGCTTTA GTATCGAGGC CAAGGTGCGT
1451 GGCTGCCCCC AGGAGAAGGA GCAGTCTTTC ACTATCAAGC CTGTGGGCTT
1501 TAAGGACAGC CTCACCGTCC AGGTGACCTT CCACTGTGAC TGTGCCCTGCC
1551 AGGCCTTTGC CCAGCCTTCC AGCCACGCT GCAACAATGG GAACGGGACT
1601 TTTGAGTGTG GGGTGTGCCG CTGTGACCAG GGCTGGCTGG GGTCCATGTG
1651 TGAGTGCTCT GAGGAGGATT ACCGACCCTC TCAGCAGGAA GAGTCAGCC
1701 CCAAGGAGGG CCAGCCCCATC TGCAGCCAGC GGGGAGAGTG CCTCTGTGGC
1751 CAGTGTGTCT GCCATAGCAG CGACTTCGGC AAGATCACTG GCAAGTACTG
1801 TGAGTGCGAT GACTTCTCCT GCGTCCGCTA CAAAGGGGAG ATGTGTTCCG
1851 GCCATGGGCA ATGTAAGTGT GGGGACTGCG TGTGTGACTC GGACTGGACT
1901 GGCTACTACT GCAACTGTAC TACACGCACT GACACCTGCA TGTCCACCAA
1951 TGGGCTGCTG TGCAGCGGCC GGGGCAACTG CGAGTCCGCG AGCTGTGTGT
2001 GCGTCCAGCC AGGCTCCTAT GGAGACACCT GTGAGAAGTG CCCCACCTGC
2051 CCAGATGCCT GCTCCTTTAA GAAGGAGTGT GTGGAGTGA AGAAGTTCAA

FIG. 1C

RECTIFIED SHEET (RULE 91)

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2101 CCGGGAACG CTCCATGAAG AAAACACCTG CAGCCGCTAC TGCCGGGATG
2151 ACATCGAGCA GGTGAAGAG CTGACGGATA CTGGCAAAA CGCCGTGAAT
2201 TGTACCTACA AGAACGAGGA TGA CTGTGTC GTCAGATTCC AGTACTACGA
2251 AGACACCAGT GGGAGGGCAG TCCTCTATGT GGTGGAAGAG CCTGAGTGTC
2301 CCAAGGTCC TGATATCCTG GTGGTACTGC TGTCA GTGAT GGGGGCCATC
2351 CTGCTCAT TG GCCTTGCTAC TCTGCTCATC TGGAAGCTAC TCATCACCAT
2401 CCATGACCGG AAGGAATTG CTA AATTGA GGAAGAACGA GCCAGAGCTA
2451 AGTGGGACAC AGCAACAAC CCGCTGTATA AAGAGGCCAC CTCCACCTTC
2501 ACCAATATCA CGTACCGAGG AACTTAATGA

FIG. 1D

RECTIFIED SHEET (RULE 91)

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1 ATAACAATT CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCTCG
51 AAATTAAACC TCACTAAAGG GAACAAAAGC TGGAGCTCCA CCGTGCGCGG
101 CCGCTCTAGA ACTAGTGGAT CCCCCGGGCT GCAGGAATTC GCGCCGTCGA
151 CGCGGGGAC AGGATGCGAG CGCAGTGGCC GGGACAACTC TGGGCCGCTC
201 TGCTGGCGCT GGGGGCGCTG GCGGGCGTGT TTGTGGAGA GTCCAACATC
251 TGTACCAACAC GAGGCGTGAA CTCCTGCCAG CAGTGTCTGG CTGTGAGTCC
301 TGTGTGTGCC TGGTGCTCAG ATGAGACTTT GTCTCAGGCC TCACCCCGAT
351 GTAACCTGAA GGAGAACCTG CTGAAGGACA ATTGTGCTCC AGAGTCTATT
401 GAGTTCCCAG TCAGTGAGGC CCAGATCCTG GAGGCTAGGC CACTCAGCAG
451 CAAGGGCTCT GGAAGCAGCG CCCAGATCAC TCAAGTCAGC CCTCAGAGGA
501 TTGCCCTTCG ACTACGGCCA GATGATTGGA AGATCTTCTC ACTTCAAGTG
551 CGGCAGGTGG AGGATTACCC CGTGGACATC TACTACTTGA TGGACCTGTC
601 TTTCTCCATG AAGGATGATC TGTCCAGCAT CCAGACCCCTG GGTACCAAGT
651 TGGCCTCTCA GATGCGCAAG CTTACTAGCA ACCTTCGGAT TGGCTTTGGG

FIG. 2A

RECTIFIED SHEET (RULE 91)

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701 GCCTTCGTGG ACAAGCCTGT ATCGCCGTAC ATGTACATCT CCCCACCACA
751 GGCAATCAAA AACCCCTGTT ACAATATGAA GAATGCCTGC TTGCCCATGT
801 TTGGCTACAA ACACGTGCTG ACGCTAACCG ACCAGGTGC CCGCTTCAAT
851 GAAGAAGTGA AGAAACAGAG CGTGTCCCGT AATCGAGATG CCCCAGAGGG
901 CCGCTTTGAC GCCATCATGC AGGCTACAGT ATGTGATGAA AAAATTGGCT
951 GGAGGAATGA CGCATCCCAT TTGCTAGTGT TTACCACGGA TGCCAAGACC
1001 CATATTGCCC TGGATGGAAG ACTGGCAGGC ATTGTCCTGC CCAATGATGG
1051 GCACTGTCAC ATTGGCACCG ACAACCACTA CTCTGCCCTCC ACTACCATGG
1101 ACTACCCATC TCTGGGGCTG ATGACTGAGA AACTATCCCA GAAAAACATT
1151 AACTTGATCT TTGCAGTGAC TGAAAATGTC GTCAGCCCTT ACCAGAATTA
1201 TAGTGAGCTC ATTCCTGGGA CCACAGTGGG AGTCCTGTCT GATGACTCAA
1251 GCAACGTCCT CCAGCTGATT GTTGATGCTT ACGGAAAAT CCGCTCTAAA
1301 GTGGAGCTGG AAGTACGTGA CCTGCCGGAA GAACTGTCAC TGTCTTCAA
1351 TGCCACCTGC CTCAACAACG AGGTTATCCC GGGCCTCAAG TCTTGTGTGG

FIG. 2B

RECTIFIED SHEET (RULE 91)

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1401 GCCGCAAGAT TGGAGACAG GTGAGCTTTA GTATCGAGGC CAAGTGCGGT
1451 GGCTGCCCCC AGGAGAAGGA GCAGTCTTTC ACTATCAAGC CTGTGGGCTT
1501 TAAGGACAGC CTCACCGTCC AGGTGACCTT CGACTGTGAC TGTGCCCTGCC
1551 AGGCCCTTGC CCAGCCTTCC AGCCACGCT GCAACAATGG GAACGGGACT
1601 TTTGAGTGTG GGGTGTGCCG CTGTGACCAG GGCTGGCTGG GGTCCATGTG
1651 TGAGTGCTCT GAGGAGGATT ACCGACCCTC TCAGCAGGAA GAGTGCAGCC
1701 CCAAGGAGGG CCAGCCCATC TGCAGCCAGC GGGGAGAGTG CCTCTGTGGC
1751 CAGTGTGTCT GCCATAGCAG CGACTTCGGC AAGATCACTG GCAAGTACTG
1801 TGAGTGCGAT GACTTCTCCT GCGTCCGCTA CAAAGGGGAG ATGTGTTCGG
1851 GCCATGGGCA ATGTAAGTGT GGGGACTGCG TGTGTGACTC GGA CTGGACT
1901 GGCTACTACT GCAACTGTAC TACACGCACT GACACCTGCA TGTCCACCAA
1951 TGGGCTGCTG TGCAGCGGCC GGGGCAACTG CGAGTGCGGC AGCTGTGTGT
2001 GCGTCCAGCC AGGCTCCTAT GGAGACACCT GTGAGAAGTG CCCCACCTGC
2051 CCAGATGCCT GCTCCTTTAA GAAGGAGTGT GTGGAGTCTA AGAAGTTCAA

FIG. 2C

RECTIFIED SHEET (RULE 91)

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2101 CCGGGGAACG CTCCATGAAG AAAACACCTG CAGCCGGCTAC TGCCGGGATG
2151 ACATCGAGCA GGTGAAAGAG CTGACGGATA CTGGCAAAA CGCCCGCGGC
2201 CCGGTCGACT GGAGACTCAC GGAGCATGAC ATACTCACCT GTCACCTATT
2251 TAGAAGACTG AGGCAGGAAG ATAAGTTTCT GGACAGCCTA GTCTGCATAA
2301 AGACCACCCCT GTCTCAAAA GCATAAAAG GCGTGGTGA ATGCCCTGCTT
2351 AGCATATAGC CCTTGGTTGC AGGTAGTGA GTACATAGGT GAAATCTGCC
2401 GCTACCTGCT GAGGCAGCCG GTTCGGGACG TGGAGCAGCG ACACCGCGTG
2451 CGCCTGGCCG CCGGTAATGG GCTGCGGCCA GCCATCTGGG AGGAGTTCAC
2501 GCAGCGCTTC GGTGTGCCAC AGATCGGCGA GTTCTACGGC GCTACCGAGT
2551 GCAACTGAGC ATTGCCAACA TGGACGGCAA GGTTCGCAGC TGTGGGGTGC
2601 AGGCGGGCGC TGTCGGTTTC CTACGACACA AGAGCCTTCA GGCCGCCCTC
2651 ACCGCCGCTG TATTCACCCCT AGGTCGGCTC CTGCGGCTTC AACAGCCGTA
2701 TCCTCACGCA TGTGTACCCC ATCCGTCTGG TCAAGGTCAA TGAGGACACG
2751 ATGGAGCCAC TGGGGGACTC CGAGGGCCTC TGCATCCCGT GCCAGCCCGG

FIG. 2D

RECTIFIED SHEET (RULE 91)

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2801 TGAGTGTGGC CCTTGCCTGG TGCCTCGGG AGCTAGAGTC CCCACGGCCC
2851 CCACACCCAC TCAGCTTGAG TGTC AACCTC CTTCCAGGG AACCCGGCCT
2901 TTCGTGGGC AGATCAACCA GCAGACCCT CTGCGCGTT TCGATGGTTA
2951 TGTTAGTGAC AGTGCCACCA ACAAGAAGAT TGCCCCACAGC GTTTTCCGAA
3001 AGGCGATACG GCCTACCTCT CAGGTGCGGA CGCTCGTGT CGTGGCTGGG
3051 CTGGCTGTCA GACTGCAAAG CCCGGTCCCA TCTGCCCCCTC TTCCCTGCAG
3101 GTGACGTGCT AGTGATGGAC GAGCTGGGT ACATGTATTT CCGTGACCGC
3151 AGCGGGGACA CCTTCCGCTG GCGCGGAGA ACGTGTC AA CCACGGAGGT
3201 GAAGCCGGTG CTGAGCCGCC TACTGGGCCA GACGGACGTG GCTGTGTATG
3251 GGGTGGCTGT GCAGGCAAGC TGGGGACACA GGGTGGTTGT GGTGTGCAGG
3301 AGCCCCATGG AGTCCATCCA GAAGGACCT GCAGGTACAG TACCCGTGGG
3351 CCATGCACAA GGTGGAGAAC TGTGTGCTG CTGACTGGGT GGGCACTGGG
3401 TTGGGAATCC ATCCACATTC CTAATATGA ACTTCAGTCT GGGGACCCCC
3451 TTCTCAGGAT CAGAAGGCTG AAACAGGTC GACGCCGCCC GGAATTCCAT
3501 ATCAAGCTTA TCGATCC

FIG. 2E

RECTIFIED SHEET (RULE 91)

10/20

1 *QFHTGNSYD HDYAKLEINP H*REQKLELH RWRPL*N*WI PRAAGIRAVD
51 AADRMRAQWP GQLWAALLAL GALAGVVUGE SNICTTRGVN SCQQCLAVSP
101 VCAWCSEDTL SQGSPRCNLK ENLLKDNCAE ESIEFPVSEA QILEARPLSS
151 KSGSSAQIT QVSPQRIALR LRPDDSKIFS LQVRQVEDYP VDIYYLMDLS
201 FSMKDDLSSI QTLGTKLASQ MRKLTSNLRI GFAGFVDKPV SPYMYISPPQ
251 AIKNPCYNMK NACLPMFGYK HVLTLTDQVS RFNEEVKKQS VSRNRDAPEG
301 GFDAIMQATV CDEKIGWRND ASHLLVFTTD AKTHIALDGR LAGIVLPNDG
351 HCHIGTDNHY SASTMDYPS LGLMTEKLSQ KNINLIFAVT ENVVSLYQNY
401 SELIPGTTVG VLSDDSSNVL QLIVDAYGKI RSKVELEVVD LPEELSLSFN
451 ATCLNNEVIP GLKSCVGRKI GDTVFSFSIEA KVRGCPQEKE QSFTIKPVGF
501 KDSLTVQVTF DCDACQAFQ QPSSPRCNG NGTFECGVCR CDQWLGS MC
551 ECSEEDYRPS QQEECPKEG QPICSQRGEC LCGQCVCHSS DFGKITGKYC
601 ECDDFSCVRY KGEMCSGHQ CNCGDCVCDS DWTGYCNCNT TRTDTMSTN
651 GLLCSGRGNC ECGSCVCVQP GSYGDTCEKC PTCPDACSFK KECVECKFN

FIG. 3A

RECTIFIED SHEET (RULE 91)

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701 RGTLHEENTC SRYCRDDIEQ VKELTDTGKN ARGRVDWRLT EHDILTCHLF
751 RRLRQEDKFL DSLVCIKTTL SQKA*KGRGE CLLSI*PLVA GSAVHR*NLP
801 LPAEAAGSRR GAATPRAPGR G*WAAASHLG GVHAALRCAT DRRVLRRYRV
851 QLSIANMDGK VRSCGVQAGA VGFLRHKSLQ AALTAAVFTL GRLLRLQOPY
901 PHACVPHPSG QGQ*GHDGAT AGLRGPLHPV PAR*VWPLPG ASGS*SPHGP
951 HTHSA*VSTS FQGNPAFRGP DQPAGPSAAF RWLC**QCHQ QEDCPQRFPK
1001 GDTAYLSGAD ARGRGWAGCQ TAKPGPICPS SLQVTC**WT SWATCISVTA
1051 AGTPSAGAGE RVQPRR*SRC *AAYWARRTW LCMGWLCRQA GDTGWLWCAG
1101 APWSPSRRDL QVQYPWAMHK VENCVAADWV GTGLGIHPHS *Y*TSVWGTP
1151 SQDQKAENRS TPPGIRYQAY RS

RECTIFIED SHEET (RULE 91)

FIG. 3B

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1 *QFHTGNSYD HDYAKLEINP H*REQKLELH RWRPL*N*WI PRAAGIRAVD
51 AADRMRAQWP GQLWAALLAL GALAGVVGE SNICTTRGVN SCQQCLAVSP
101 VCAWCSEDTL SQGSPRCNLK ENLLKDNAP ESIEFPVSEA QILEARPLSS
151 KSGSSAQIT QVSPQRIALR LRPDDSKIFS LQVRQVEDYP VDIYYLMDLS
201 FSMKDDLSSI QTLGTKLASQ MRKLTSNLRI GFGAFVDKPV SPYMYISPPQ
251 AIKNPCYNMK NACLPMFGYK HVLTLTDQVS RFNEEVKKQS VSRNRDAPEG
301 GFDAIMQATV CDEKIGWRND ASHLLVFTTD AKTHIALDGR LAGIVLPNDG
351 HCHIGTDNHY SASTTMDYPS LGLMTEKLSQ KNINLIFAVT ENVVS LYQNY
401 SELIPGTTVG VLSDDSSNVL QLIVDAYGKI RSKVELEV RD LPEELSLSFN
451 ATCLNNEVIP GLKSCVGRKI GDTVFSFIEA KVRGCPQEKE QSFTIKPVGF
501 KDSLTVQVTF DCDCACQAF A QPSSPRCNG NGTFECGVCR CDQGWLGSMC
551 ECSEEDYRPS QQEECSPEKEG QPICSQRGEC LCGQCVCCHSS DFGKITGKYC
601 ECDDFSCVRY KGEMCSGHGQ CNGDCVCDS DWTGYCNCNCT TRTDTMSTN
651 GLLCSGRGNC ECGSCVCVQP GSYGDTCEKC PTCPDACSFK KECVECKFN

FIG. 4A

RECTIFIED SHEET (RULE 91)

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701 RGTLHEENTC SRYCRDDIEQ VKELDTGKN AVNCTYKNED DCVVRFQYYE
751 DTSGRAVLVY VEEPEC PKGP DILVVLLSVM GAILLIGLAT LLIWKLITI
801 HDRKEFAKFE EERARAKWDT ANNPLYKEAT STFTNITYRG T**

FIG. 4B

RECTIFIED SHEET (RULE 91)

14/20

55
MRAQWPGQLWAALLALGALAGVVVGESNICCTTRGVNSCQQCLAVSPVCAW 104

|||||

55
MRAQWPGQLWAALLALGALAGVVVGESNICCTTRGVNSCQQCLAVSPVCAW 104

105
CSDETLSQGSPPRCNLKENLLKDNCAPESIEFPVSEAQILEARPLSSKSGS 154

|||||

105
CSDETLSQGSPPRCNLKENLLKDNCAPESIEFPVSEAQILEARPLSSKSGS 154

155
SSAQITQVSPQRIALRLRPDDSKIFSLQVRQVEDYPVDIYYLMDLSFSMK 204

|||||

155
SSAQITQVSPQRIALRLRPDDSKIFSLQVRQVEDYPVDIYYLMDLSFSMK 204

205
DDLSSIQTLGTKLASQMRKLTSNLRIGFGAFVDKPVSPYMYISPPQAIKN 254

|||||

205
DDLSSIQTLGTKLASQMRKLTSNLRIGFGAFVDKPVSPYMYISPPQAIKN 254

255
PCYNMKNACLPMFGYKHVLT LTDQVSRFNEEVKKQSVSRNRDAPEGGFDA 304

|||||

255
PCYNMKNACLPMFGYKHVLT LTDQVSRFNEEVKKQSVSRNRDAPEGGFDA 304

FIG. 5A

RECTIFIED SHEET (RULE 91)

15/20

305
IMQATVCDEKIGWRNDASHLLVFTTDAKTHIALDGRLAGIVLPNDGHCHI 354

|||||

305
IMQATVCDEKIGWRNDASHLLVFTTDAKTHIALDGRLAGIVLPNDGHCHI 354

355
GTDNHYSASTTMDYPSLGLMTEKLSQKNINLIFAVTENVVSLYQNYSELI 404

|||||

355
GTDNHYSASTTMDYPSLGLMTEKLSQKNINLIFAVTENVVSLYQNYSELI 404

405
PGTTVGVLSDSSNVLQLIVDAYGKIRSKVELEVRLPEELSLSFNATCL 454

|||||

405
PGTTVGVLSDSSNVLQLIVDAYGKIRSKVELEVRLPEELSLSFNATCL 454

455
NNEVIPGLKSCVGRKIGDTVFSFSIEAKVRGCPQEKEQSFTIKPVGFKDSL 504

|||||

455
NNEVIPGLKSCVGRKIGDTVFSFSIEAKVRGCPQEKEQSFTIKPVGFKDSL 504

505
TVQVTFDCDCACQAFAPSSPRCNGNGTFECGVCRCQGWLGSMCECSE 554

|||||

505
TVQVTFDCDCACQAFAPSSPRCNGNGTFECGVCRCQGWLGSMCECSE 554

FIG. 5B

RECTIFIED SHEET (RULE 91)

16/20

555
EDYRPSQQEECS PKEGQPICSQRGECLCGQCVCHSSDFGKITGKYCECDD 604

|||||

555
EDYRPSQQEECS PKEGQPICSQRGECLCGQCVCHSSDFGKITGKYCECDD 604

605
FSCVRYKGEMCSGHGQCNCGDCVCDSDWTGYCNCCTTRTDTCMSTNGLLC 654

|||||

605
FSCVRYKGEMCSGHGQCNCGDCVCDSDWTGYCNCCTTRTDTCMSTNGLLC 654

655
SGRGNCECGSCVCVQPGSYGDTCEKCPTCPDACSFKKECVECKKFNRGTL 704

|||||

655
SGRGNCECGSCVCVQPGSYGDTCEKCPTCPDACSFKKECVECKKFNRGTL 704

705
HEENTCSRYCRDDIEQVKELTDTGKNAVNTYKNEDDCVVRFQYYEDTSG 754

|||||

. | : . . | . .

705
HEENTCSRYCRDDIEQVKELTDTGKNA.....RGRVDWRLTEHDIL 745

755 RAVLYVVEEPEC PKGPDILVLLSVMGA 782

. | : . | . | . | | . : . . : .

746 TCHLFRRRLRQE.DKFLDSLVCIKTTLSQ 772

FIG. 5C

RECTIFIED SHEET (RULE 91)

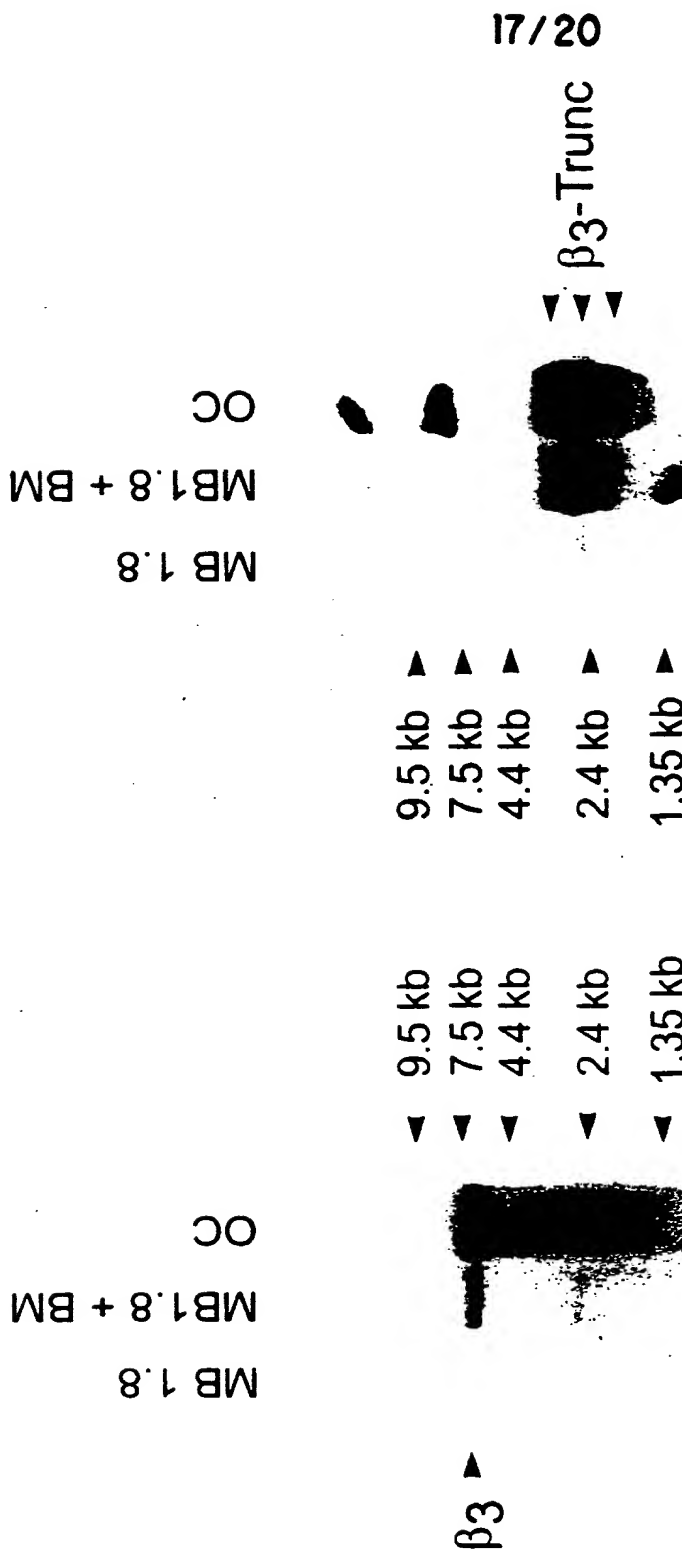


FIG. 6B

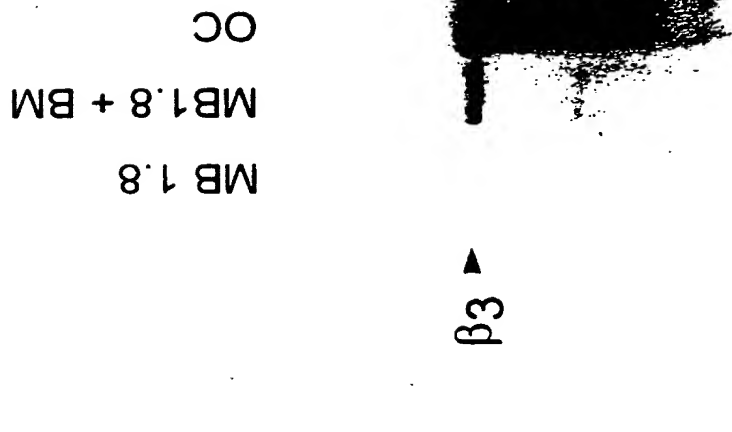


FIG. 6A

SUBSTITUTE SHEET (RULE 26)

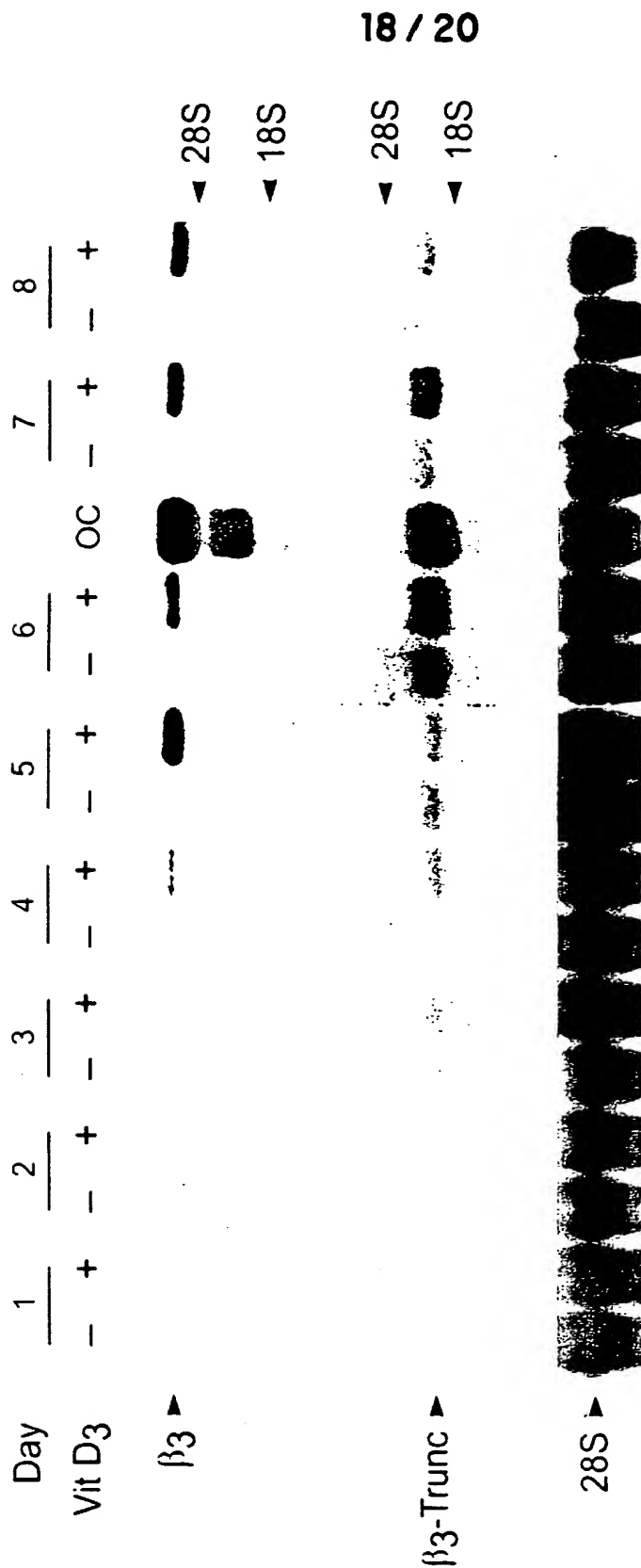


FIG. 7

SUBSTITUTE SHEET (RULE 26)

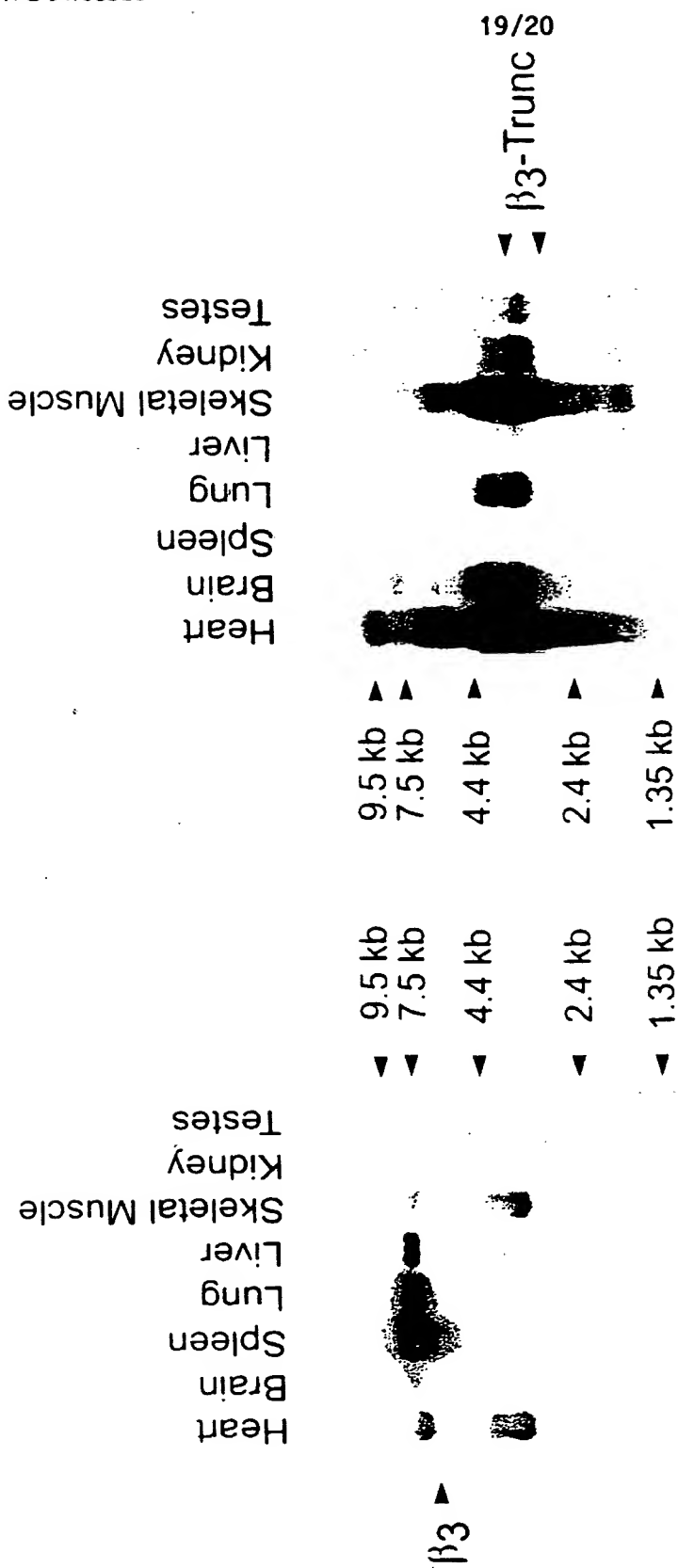


FIG.8B

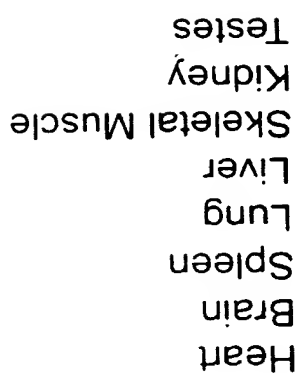


FIG.8A

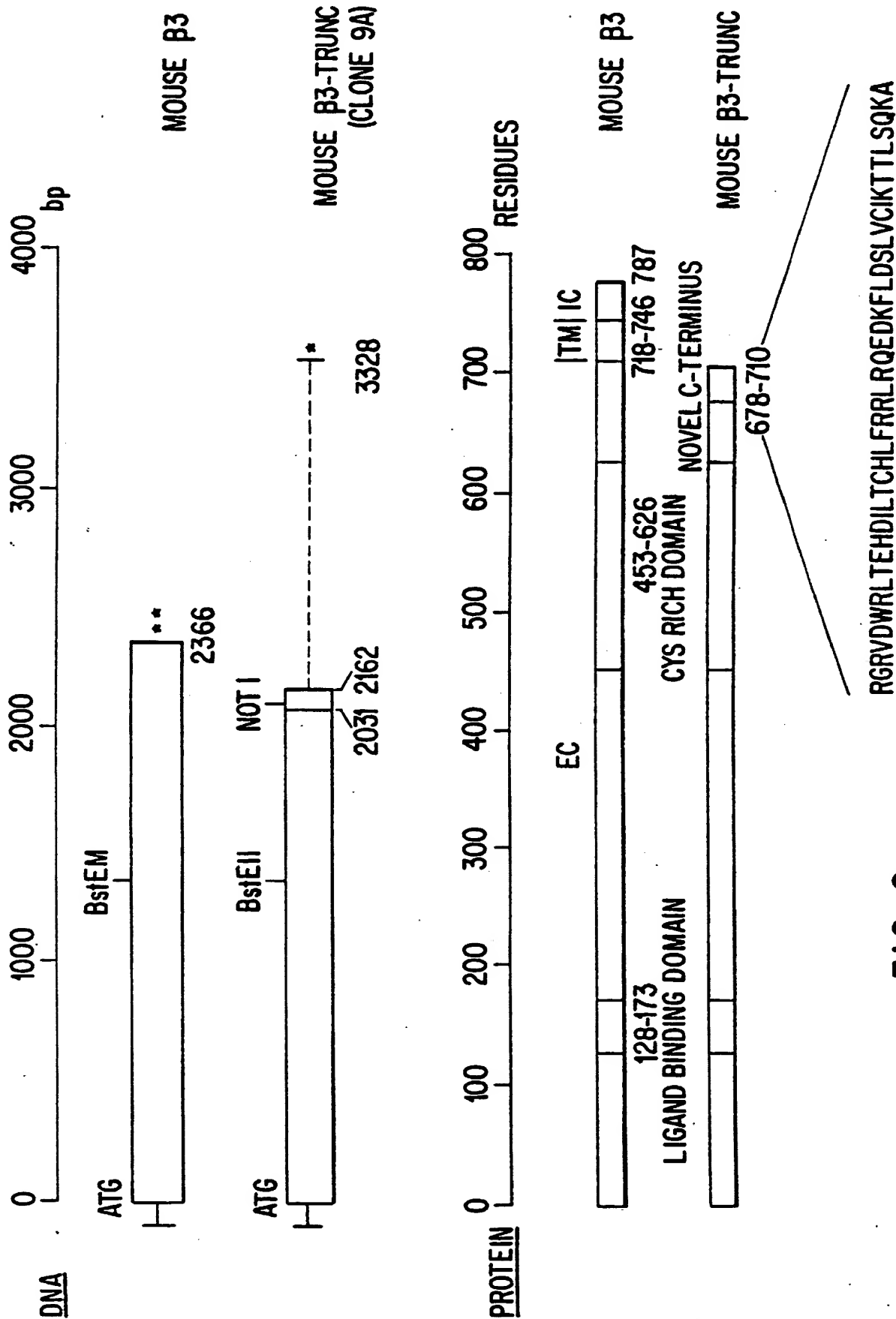
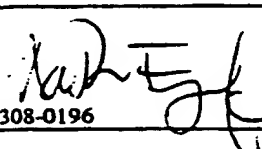


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
T/US96/13805

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : C12N 15/12, 15/63, 15/70, 15/79; C07K 14/705 US CL : 536/23.5, 530/350, 435/69.1, 320.1, 240.2, 252.3 According to International Patent Classification (IPC) or to both national classification and IPC																				
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/23.5, 530/350, 435/69.1, 320.1, 240.2, 252.3 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, Medline, Hcaplus search terms: beta 3, integrin#, murine, mouse																				
C. DOCUMENTS CONSIDERED TO BE RELEVANT																				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																		
Y	CIEUTAT, A.-M. et al. A Comparative Analysis of cDNA-Derived Sequences for Rat and Mouse β_3 Integrins (GPIIIa) with their Human Counterpart. Biochem. Biophys. Res. Comm. 15 June 1993. Vol. 193, No. 2, pages 771-778, especially pages 774-775.	1-20																		
Y	DJAFFAR, I. et al. A New Alternative Transcript Encodes a 60 kDa Truncated Form of Integrin β_3 . Biochem. J. 15 May 1994. Vol. 300, Pt. 1, pages 69-74, especially page 72.	12-20																		
Y	FITZGERALD, L.A. et al. Protein Sequence of Endothelial Glycoprotein IIIa Derived from a cDNA Clone. J. Biol. Chem. 25 March 1987. Vol. 262, No. 9, pages 3936-3939, especially page 3937.	1-11																		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																				
<table border="0"><tr><td>* Special categories of cited documents:</td><td>*T</td><td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td></tr><tr><td>*A* document defining the general state of the art which is not considered to be of particular relevance</td><td>*X*</td><td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td></tr><tr><td>*E* earlier document published on or after the international filing date</td><td>*Y*</td><td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td></tr><tr><td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td><td>*&*</td><td>document member of the same patent family</td></tr><tr><td>*O* document referring to an oral disclosure, use, exhibition or other means</td><td></td><td></td></tr><tr><td>*P* document published prior to the international filing date but later than the priority date claimed</td><td></td><td></td></tr></table>			* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*&*	document member of the same patent family	*O* document referring to an oral disclosure, use, exhibition or other means			*P* document published prior to the international filing date but later than the priority date claimed		
* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																		
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																		
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																		
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*&*	document member of the same patent family																		
O document referring to an oral disclosure, use, exhibition or other means																				
P document published prior to the international filing date but later than the priority date claimed																				
Date of the actual completion of the international search 27 OCTOBER 1996		Date of mailing of the international search report 07 NOV 1996																		
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer KAREN E. BROWN  Telephone No. (703) 308-0196																		

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13805

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VAN KUPPEVELT, T.H.M.S.M. et al. An Alternative Cytoplasmic Domain of the Integrin β_3 Subunit. Proc. Natl. Acad. Sci. USA. July 1989. Vol. 86, No. 14, pages 5415-5418, especially page 5416.	12-20
Y	US 5,391,704 A (McMILLAN ET AL.) 21 February 1995, column 19, lines 26-54.	6-11, 17-20

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*